Notes from the Lab:
The Latest Bee Science Distilled

by Scott McArt

How Tropilaelaps mites reduce foraging efficiency of adult honey bees

While the varroa mite is the most important and widespread pest of honey bees across the world, a different mite, *Tropilaelaps mercedesae*, may be a bigger threat. *Tropilaelaps* (Photo 1) is smaller than varroa, has a faster reproductive rate, a shorter phoretic phase (i.e., the phase when mite is on adult bees and is most easily controlled), efficiently vectors viruses including Deformed Wing Virus (DWV), and it moves on bees and in colonies like an acrobat. It's bad news!

Thankfully, *Tropilaelaps* is still only found in Asia. It made a host switch from the Giant honey bee (*Apis dorsata*) to the European honey bee (*Apis mellifera*), which is now commonly kept by beekeepers in some parts of Asia. Because of its very recent host switch onto the European honey bee, the two haven't coevolved together in nature, which means European honey bees don't have coevolved defenses against the mite. This same lack of coevolved defenses is true for varroa, which made a host switch from *Apis cerana* to *A. mellifera* in the mid-1900s. We all see how that unfortunate dynamic for *A. mellifera* is playing out right now.

Hopefully *Tropilaelaps* will stay in Asia and we won't have to worry about it in North America. But we were also hopeful varroa would stay in Asia until it started spreading throughout the world, eventually showing up in North America in 1987. Hence, it's probably a good idea for us to start understanding more about *Tropilaelaps* so we can more effectively grapple with it if (hopefully not when!) it arrives in our backyards.

So, what does *Tropilaelaps* do to bees? We know it wounds developing larvae/pupae and transmits viruses, sometimes overwhelming a

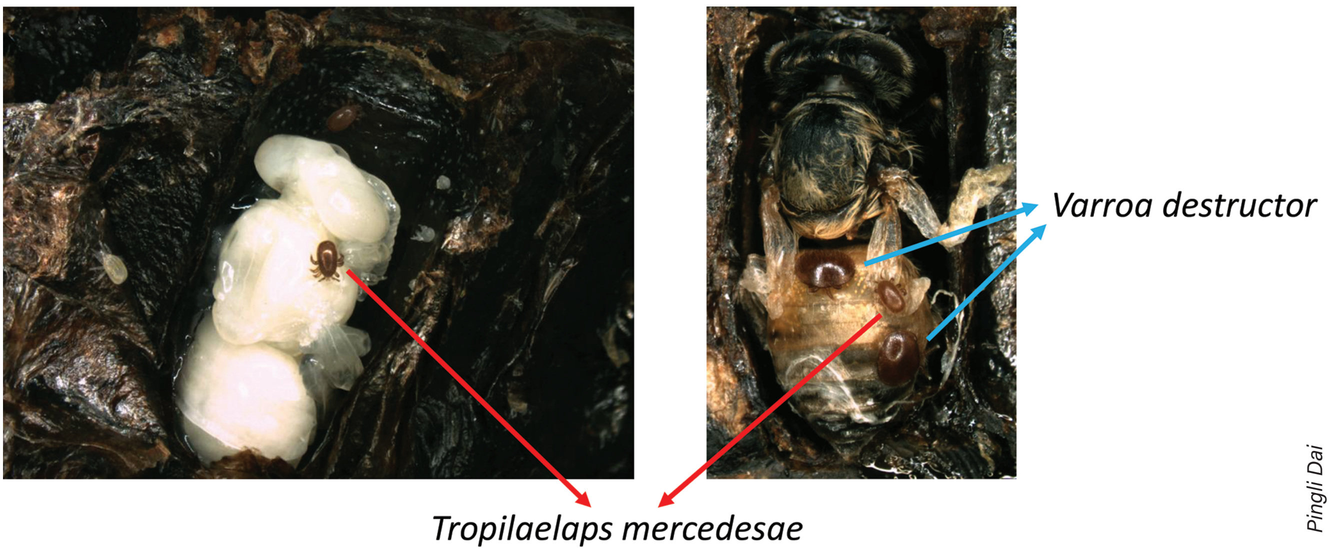

Photo 1. Apis mellifera pupa *(left) and newly emerged adult (right) infested with* Tropilaelaps mercedesae *(red arrows) and* Varroa destructor *(blue arrows)*

Photo 2. *Flight mill used to test the flight ability of tethered forager bees*

Photo 3. Apis mellifera *infested with* Tropilaelaps mercedesae *used to assess proboscis extension reflex (PER), a measure of olfactory learning*

colony with sickness. But what about impacts on foraging adults, who gather all the food for colonies? Does *Tropilaelaps* impact olfactory learning, flight ability, or homing ability of foragers? And what if we look at gene expression of foragers, can that tell us something about how the bees are stressed? These are the topics for our forty-fifth Notes from the Lab, where we summarize ***"Tropilaelaps mercedesae*** **parasitism changes behavior and gene expression in honey bee workers,"** written by Jing Gao and colleagues and published in the journal ***PLoS Pathogens*** **[2021].**

For their study, Gao and colleagues first needed to create colonies that weren't infested with *Tropilaelaps*. To do this, they took advantage of the fact that *Tropilaelaps* can only survive for two or three days when there are only adult bees around; they caged queens for one month and removed all brood from the experimental colonies. Next, they released the queen and let her start laying, then collected frames of 5th-instar larvae. For treatment bees, one foundress of *Tropilaelaps* was introduced into a newly sealed brood cell with a 5th-instar larva. Control bees were obtained from brood cells that weren't infested with *Tropilaelaps*. A total of 10 brood combs (five infested combs and five controls) were placed in an incubator and newly emerged adults (*Tropilaelaps* treatment or control) were placed in cages with 30 bees as a group. The adult bees were supplied with syrup and fresh pollen, and the cages were maintained in an incubator.

To measure flight ability, infested and non-infested worker bees were tested 15 days post-emergence on a modified flight mill, which is essentially a small treadmill for bees (Photo 2). Sensors recorded mean velocity, flight duration, and flight distance for ~120 control vs. treatment bees.

To measure homing ability, 300 infested and non-infested bees were randomly collected from brood combs, marked with different colors painted on the thorax, and placed into three non-infested colonies. After three days, all bees were released from approximately 50 m away, and homing time and number of bees successfully returning to the hive were recorded.

To measure responsiveness to sucrose and olfactory learning, bees were assessed for their proboscis extension reflex (PER) by securing them individually in 1.5 mL tubes with their antennae and mouthparts free (Photo 3). Infested vs. non-infested bees were tested for their response to 30% sucrose solutions at 1, 5, 10 and 15 days after emergence. Bees that responded after 15 days were further tested for olfactory learning. For this test, a common floral scent (linalool) was provided at the same time as the sucrose and bees were offered the scent and sucrose several times. Then, only linalool was provided and the infested vs. non-infested bees were monitored to see if they had learned to associate the scent with a reward by extending their proboscis.

Finally, to understand what might be underlying the different behaviors of infested vs. non-infested bees, the heads of bees that responded differently to sucrose and olfactory learning behavior were analyzed for gene expression using RNA sequencing.

So, what did they find? Did the flight ability of bees infested with

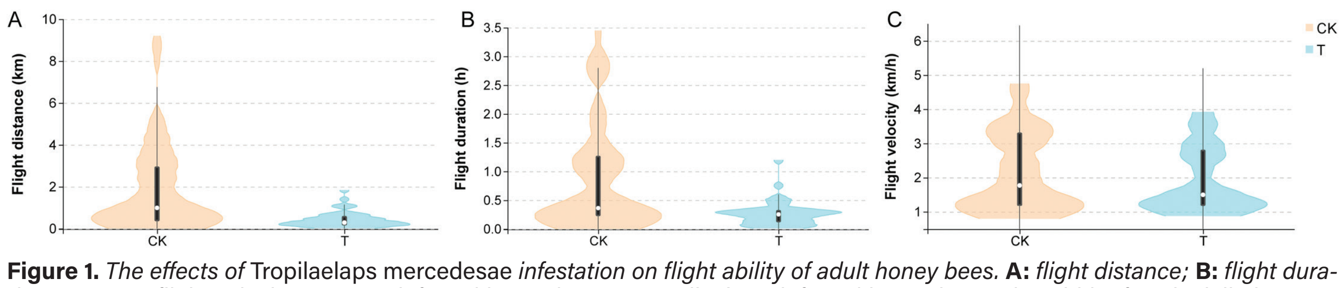

Figure 1. *The effects of* Tropilaelaps mercedesae *infestation on flight ability of adult honey bees.* **A:** *flight distance;* **B:** *flight duration;* **C:** *mean flight velocity. CK: non-infested honey bees; T:* Tropilaelaps*-infested honey bees. The width of each violin box represents the density of the data values, white dots represent the median values, and the upper and lower edges of the black thick line represent quartiles. The upper and lower ends of the thin line represent the maximum and minimum values of non-outlier data.*

***Tropilaelaps* differ from non-infested bees?** Yes. As can be seen in Figure 1, non-infested bees flew more than three times longer (panel A) and almost four times as far (panel B) as *Tropilaelaps*-infested bees. There was no difference in flight velocity (panel C).

What about the homing ability of bees? Was that impacted by *Tropilaelaps* infestation? Yes. As can be seen in Figure 2, it took about 50% longer for *Tropilaelaps*-infested bees compared to non-infested control bees to return to their colonies. This result was consistent across each of the three colonies containing *Tropilaelaps*-infested bees (T1, T2, T3) or non-infested control bees (CK1, CK2, CK3).

Was olfactory learning impacted by *Tropilaelaps* infestation and if so, why? While there were no differences in responsiveness to sucrose up to 10 days post-emergence, *Tropilaelaps*-infested bees were less likely than non-infested control bees to respond to sucrose at 15 days post-emergence. In addition, *Tropilaelaps*-infested bees were less likely to learn to associate the floral odor (linalool) with a sucrose reward.

To try and gain insight into the reasons for different olfactory responses to sucrose in *Tropilaelaps*-infested vs. non-infested bees, the authors conducted RNA sequencing on the heads of infested vs. non-infested bees. They made three major comparisons: (1) infested vs. non-infested bees that did not respond to sucrose (TSN vs. CKSN); (2) infested vs. non-infested bees that did not respond to floral odor (TN vs. CKN); and (3) infested vs. non-infested bees that each responded to sucrose (TL vs. CKL). Several genes were differentially expressed in each of the comparisons (Figure 3, panels A and B). The major take-away message from these results is that genes involved in immune function and carbohydrate transport and metabolism were significantly different between infested and non-infested bees (Figure 3, panel C). This is interesting because genes that function in cell adhesion play an essential role in olfactory sensing (i.e., the ability to smell) in honey bees.

Overall, the study by Gao and colleagues shows that *Tropilaelaps mercedesae* can have major impacts on adult foraging honey bees. Combined with their propensity to increase virus transmission and lower immunity, this is one more reason why *Tropilaelaps* mites are bad news for honey bee colonies! With apologies to the

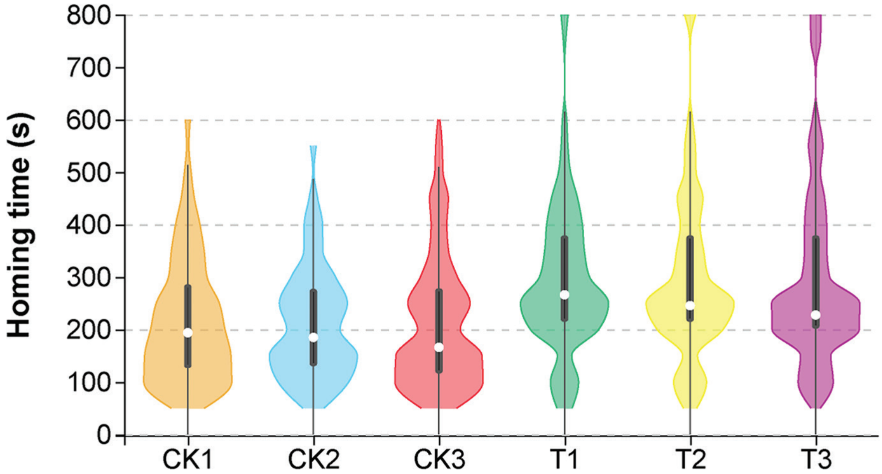

Figure 2. *The effect of* Tropilaelaps mercedesae *infestation on homing ability of adult honey bees. Violin plot shows the homing time in control bees and* Tropilaelaps-*infested bees. CK: non-infested honey bees; T:* Tropilaelaps-*infested honey bees. Description of violin plot parameters is the same as Figure 1.*

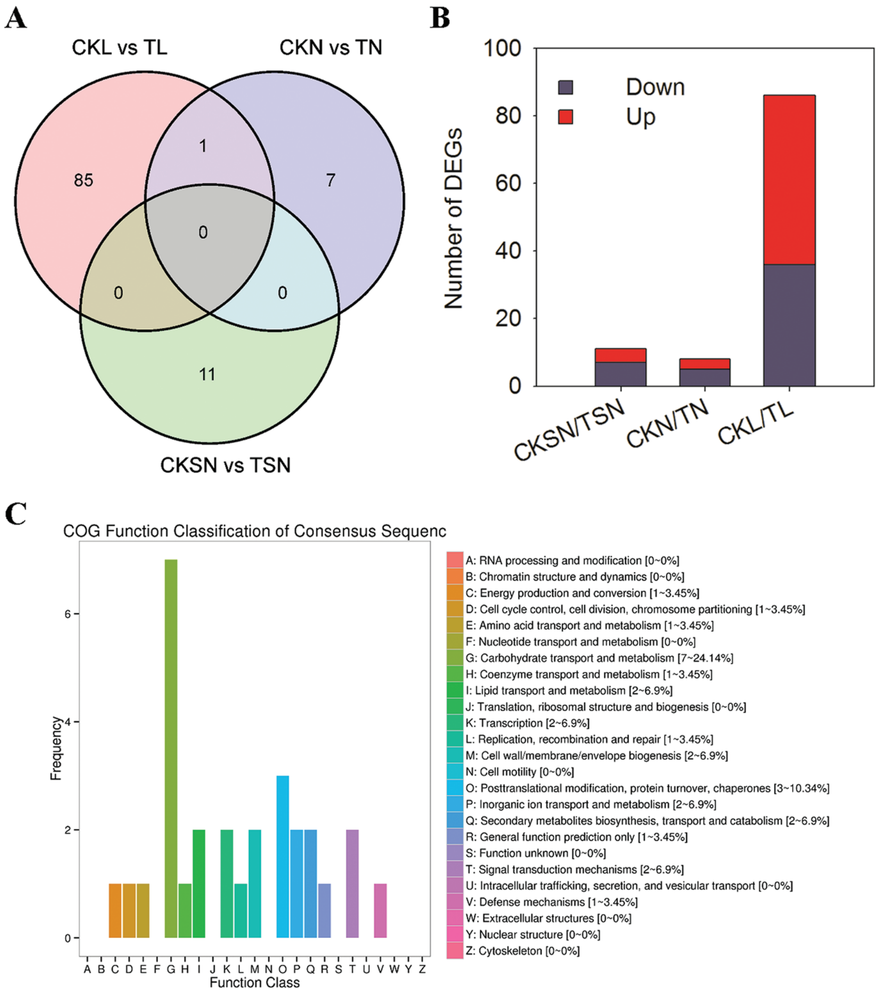

Figure 3. *The differentially expressed genes (DEGs) between healthy bees and* Tropilaelaps-*infested bees.* **A:** *Venn diagram of DEGs between bees with and without olfactory learning ability in both the* Tropilaelaps-*treated groups (TL, TN, TSN) and the non-infested control groups (CKL, CKN, CKSN).* **B:** *Number of DEGs in each comparison of* Tropilaelaps-*infested bees vs. non-infested control bees. Up-regulated and down-regulated means that these genes exhibited higher or lower expression in the infested group compared to control group.* **C:** *Clusters of Orthologous Groups (COG) Function Classification of DEGs that were up-regulated and down-regulated.*

hard-working Asian beekeepers who are trying their best to manage *Tropilaelaps*, I sincerely hope these mites stay in Asia and don't make the jump to North America. If they do arrive in our backyard, beekeeping on our continent is going to get a lot more complicated.

Until next time, bee well and do good work.

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REFERENCE:

Gao, J., Ma, S., Wang, X., Yang, Y., Luo, Q., Wang, X., Liu, F., Wang, Q., Fu, Z., Diao, Q. and P. Dai. 2021. *Tropilaelaps mercedesae* parasitism changes behavior and gene expression in honey bee workers. *PLoS Pathogens* 17(7):e1009684. **https://doi.org/10.1371/journal.ppat.1009684**

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